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Definition of the heterocyclic pharmacophore of bacterial methionyl tRNA synthetase inhibitors: potent antibacterially active non-quinolone analogues

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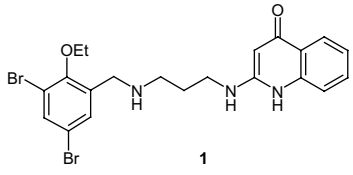
Abstract—Potent inhibitors of bacterial methionyl tRNA synthetase (MRS) have previously been reported. Through SAR of the quinolone moiety, the right hand side pharmacophore for MRS inhibition has now been defined as an NH–C–NH functionality in the context of a bicyclic heteroaromatic system. Potent antibacterial fused-pyrimidone and fused-imidazole analogues have been obtained and enantioselective activity demonstrated. Compound **46** demonstrated very good antibacterial activity against panels of antibiotic-resistant staphylococci and enterococci.

The search for new antibacterial agents acting at novel molecular targets is driven by the steadily increasing incidence of bacterial resistance to established antibiotic classes. In this context, inhibitors of bacterial aminoacyl tRNA synthetases, essential enzymes in protein biosynthesis, have attracted interest. This enzyme class is clinically validated by the topical antibiotic mupirocin (marketed as Bactroban®), which acts by inhibition of bacterial isoleucyl tRNA synthetase.

We have reported potent inhibitors of *Staphylococcus aureus* methionyl tRNA synthetase (MRS) with excellent antibacterial activity against staphylococci and enterococci.[1–3] This series of compounds, such as **1**, has been characterised by a 2-aminoquinolone right hand side. One disadvantage of these molecules is their low membrane permeability, thought to be due to the aminoquinolone moiety. Here we describe the determination of the essential right hand side pharmacophore and the identification of potent non-quinolone inhibitors.



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Analogues in which the exocyclic amine was replaced by oxygen or sulfur were prepared as shown in Scheme 1. Reaction of the *p*-methoxybenzyloxy-quinoline **2**[3] with an appropriately protected alcohol or thiol, followed by standard elaboration, afforded **4** and **5**. These compounds were tested in the standard *S. aureus* MRS acylation assay.[1] They were found to have significantly reduced potency with IC_{50} values of 38 and 680 nM, respectively, compared to 16 nM for the direct NH analogue. Compounds **4** and **5** also had very poor antibacterial activity against *S. aureus* Oxford with MIC values of 32 and >64 μg/mL. The exocyclic NH thus plays an important role in the MRS inhibitors.

The role of the functionality in the pyridone ring was explored by comparing the quinolone **6** with analogues locked in the keto **7** and enol **8** tautomers as well as the

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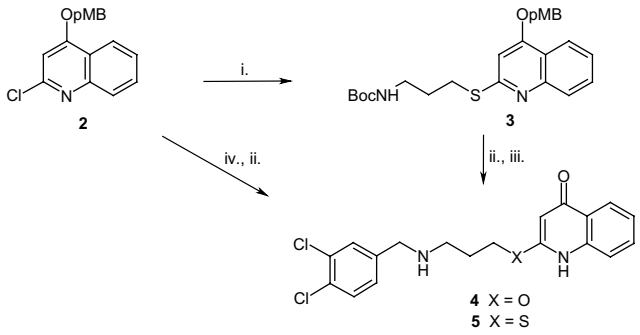
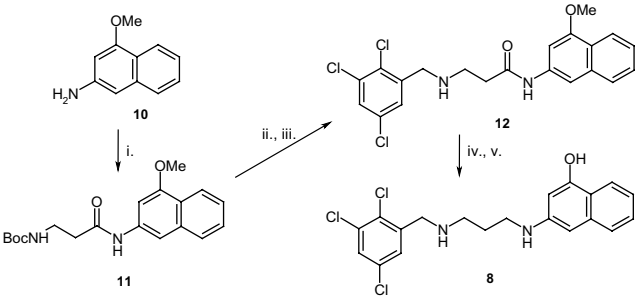


Scheme 1. Reagents and conditions: (i) $BocNH(CH_2)_3SH/NaH/THF$; (ii) TFA; (iii) 3,4-diCl-benzaldehyde/$NaCNBH_3$/AcOH/MeOH; (iv) 3,4-diCl-benzyl-$NH(CH_2)_3OH/NaH/THF/\Delta$.

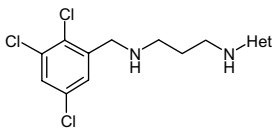


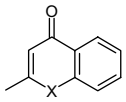
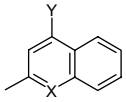
Scheme 2. Reagents and conditions: (i) $BocNH(CH_2)_2CO_2H$/EDC/HOBt/DMF; (ii) TFA/DCM; (iii) 2,3,5-tri-Cl-benzaldehyde/$NaCNBH_3$/NaOMe/MeOH; (iv) $AlCl_3/LiAlH_4$/THF/−30 °C; (v) BBr_3/dichloroethane/Δ.

deoxygenated compound **9**. Compounds **7** and **9** were prepared by the standard amine displacement reaction with 2-methanesulfanylchromone or 2-chloroquinoline, whilst **8** was synthesised via an amide coupling strategy as shown in Scheme 2.

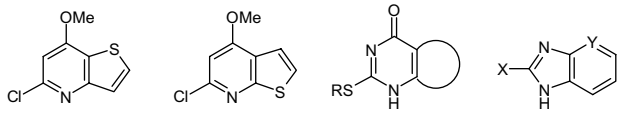
The enzyme inhibition of compounds **7**–**9** along with the quinolone **6**, are reported in Table 1. All the analogues were substantially less potent than the quinolone, indicating that the endocyclic NH also plays a key role in inhibitor binding to MRS.

Table 1. MRS inhibition of non-N–H containing quinolone analogues



No.	Het	X	Y	IC_{50} (nM) *S. aureus* MRS
6		NH	—	<3
7		O	—	1000
8		CH	OH	1800
9		N	H	100

Further analogues were prepared maintaining the crucial NH–C–NH unit. Two thienopyridones **19** and **20** were prepared by reaction of *N*-(3,5-dibromobenzyl)-1,3-diaminopropane with the chloropyridines **13** and **14**, followed by standard acid hydrolysis.[4]



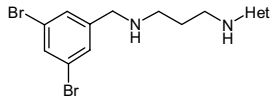
An array of more structurally diverse analogues was also prepared. Suitable NH-containing heterocycles bearing a leaving group at the 2-position were either reacted with *N*-(3,5-dibromobenzyl)-1,3-diaminopropane, or in some cases with 1,3-diaminopropane and the product then reductively alkylated with 3,5-dibromobenzaldehyde. Pyrimidone-type analogues were prepared from 2-alkylthio starting materials **15** and imidazole-type analogues from 2-halo **16** or 2-alkylthio **17** starting materials. Either excess amine or diisopropylethylamine was used as base. The cyanoindole **28** was prepared by displacement on a 2-chloroindole intermediate[5] whilst the urea **29** was prepared by reaction with phenyl isocyanate.

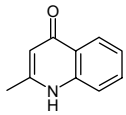
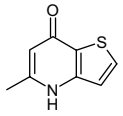
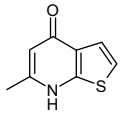
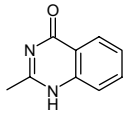
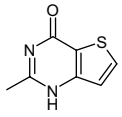
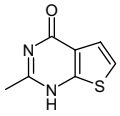
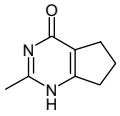
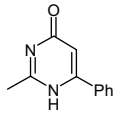
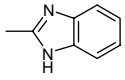
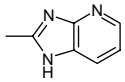
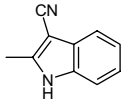
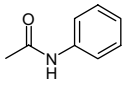
Key analogues are detailed in Table 2, along with their MRS IC_{50} values and antibacterial activity against *S. aureus* and *Enterococcus faecalis*. Most of the fused heteroaromatic analogues afforded potent enzyme inhibition and very good antibacterial activity. A large reduction in potency was observed when one ring was saturated (**24**), when the second ring was pendant rather than fused (**25**), or when the first ring was not present (**29**).

The poor inhibition of the thienopyrimidone **23** is in notable contrast to that of the other bicyclic heteroaromatics. However, the low potency of **23** may be attributable to a low concentration of the desired 1-NH tautomer of this compound. Calculation of the energy of the 1-H and 3-H tautomers of the 2-NHMe derivatives of the heterocycles of **18**–**23** showed that the thienopyrimidone **23** had a significantly higher preference for the 3-H isomer compared to all the other structures.[6] Thus an NH–C–NH functionality presented in the context of a bicyclic heteroaromatic appears to define the left hand side pharmacophore for bacterial MRS inhibition.

Further elaboration was focused on the active heterocycles that were more different from the quinolone, namely the benzopyrimidone (BP) of **21**, the thienopyrimidone (TP) of **22**, the benzimidazole (BI) of **26** and the azabenzimidazole (AB) of **27**. Left hand side analogues were prepared by the standard reductive amination methodology[1,2] with aldehydes (Table 3) or cyclic ketones (Table 4).

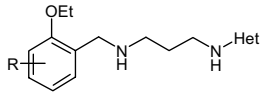
Table 2. MRS inhibition and antibacterial activity of N–H containing right hand side analogues



	Het	IC_{50} (nM)	MIC (μg/mL)	
		S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
18		<3	0.25	⩽0.06
19		4	0.13	⩽0.06
20		6.2	0.25	⩽0.06
21		8.1	0.25	⩽0.06
22		3.9	0.5	⩽0.06
23		150	>64	32
24		580	>64	>64
25		>1000	>64	>64
26		29	1	0.13
27		5.0	0.5	0.25
28		54	0.5	0.25
29		330	>64	8

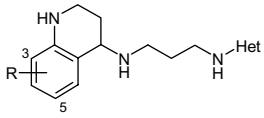
In the benzyl series, the 2,3,5-substituted analogues had potent MRS inhibition and good antibacterial activity (Table 3). The benzimidazole analogues, compounds **32**, **35**, **38** and **41** were clearly not as good as the pyrimidones. From the tetrahydroquinoline series there were also a number of analogues derived from the four

Table 3. MRS inhibition and antibacterial activity of 2,3,5-trisubstituted phenyl analogues



	R	Het	IC_{50} (nM)	MIC (μg/mL)	
			S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
30	3,5-diCl	BP	<3	0.13	⩽0.06
31	3,5-diCl	TP	<3	0.25	0.13
32	3,5-diCl	BI	<3	2	0.5
33	3-Br,5-Cl	BP	<3	0.13	⩽0.06
34	3-Br,5-Cl	TP	<3	0.13	0.13
35	3-Br,5-Cl	BI	3.8	1	0.5
36	3,5-diBr	BP	9.7	0.13	⩽0.06
37	3,5-diBr	TP	4.9	0.13	⩽0.06
38	3,5-diBr	BI	17	1	0.25
39	3-Br,5-OMe	BP	7.0	0.13	⩽0.06
40	3-Br,5-OMe	TP	3.3	0.25	⩽0.06
41	3-Br,5-OMe	BI	17	1	0.13

Table 4. MRS inhibition and antibacterial activity of tetrahydroquinoline analogues



	R	Het	IC_{50} (nM)	MIC (μg/mL)	
			S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
42	3-Cl,5-Br	BP	8.2	⩽0.06	⩽0.06
43	3-Cl,5-Br	TP	<3	⩽0.06	⩽0.06
44	3,5-diBr	TP	13	⩽0.06	⩽0.06
45	3,5-diBr	BI	13	0.25	⩽0.06
46	3,5-diBr	AB	11	⩽0.06	⩽0.06
47	3-I,5-Et	BP	17	⩽0.06	0.25
48	3-I,5-Et	TP	<3	⩽0.06	0.5
49	3-I,5-Et	BI	16	0.5	1
50	3-I,5-Et	AB	18	0.25	1

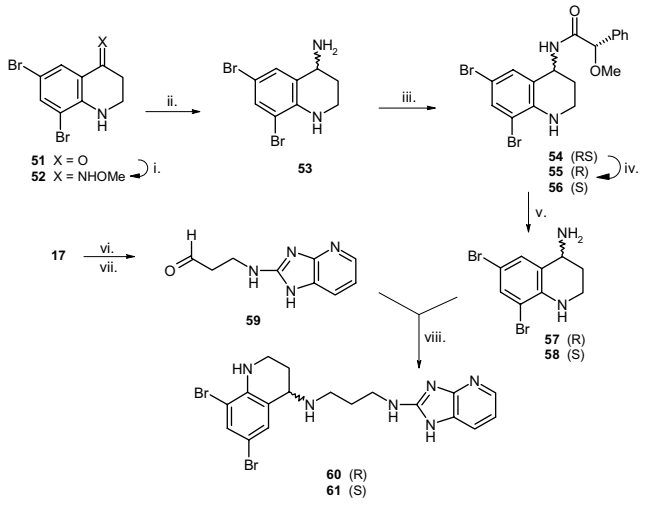
selected heterocycles, which showed excellent antibacterial activity against *S. aureus* and *E. faecalis* (Table 4).

Inhibitors containing the new right hand side heterocycles all retained selectivity for the bacterial enzyme, with none of the compounds giving significant inhibition of mammalian (rat liver) MRS up to the highest concentration tested (either 1 or 10 μM).

Compound **46** was tested against a wider range of clinical isolates of *S. aureus*, *Staphylococcus epidermidis*, *E. faecalis* and *Enterococcus faecium* to determine MIC90 values (the concentration required to inhibit 90% of the organisms). The panels of isolates included a large proportion of organisms resistant to various clinical antibiotics.[1] Very good activity was seen against all the organisms, with all MIC90 values at ⩽1 μg/mL (MIC90's: *S. aureus*, 1 μg/mL; *S. epidermidis*, 0.5 μg/mL; *E. faecalis*, 0.06 μg/mL; and *E. faecium* 0.03 μg/mL).

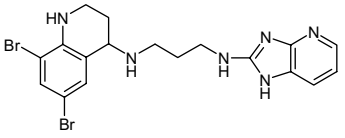
In view of the encouraging properties of **46**, the individual enantiomers were targeted, as the tetrahydroquinolines had only been prepared as racemates. The synthesis of the enantiomers is shown in Scheme 3. The diastereoisomers of amide **54** were separated chromatographically and the absolute configuration was assigned via an X-ray crystal structure of isomer **56**.[7] The separate diastereomers were then deprotected to give the enantiomeric amines **57** and **58**. A reverse coupling strategy was used, whereby reductive alkylation of the tetrahydroquinoline amines was effected with the aldehyde **59**. The enantiomeric excess (ee) of the final products was determined by chiral capillary zone electrophoresis (cze) to be 98.4% for the (*R*)-isomer **60** and 99.0% for the (*S*)-isomer **61**.[8]

The enantiomers **60** and **61** were assayed in the usual way (Table 5). The (*R*)-enantiomer **60** was found to be the more active isomer with a lower IC_{50} value and



Scheme 3. Reagents and conditions: (i) $MeONH_2{\cdot}HCl/NaOAc/EtOH/H_2O/\Delta$; (ii) $ZrCl_4/LiBH_4/THF$; (iii) (*S*)-PhCH(OMe)CO_2H/EDC/HOAt/*N*-Me-morpholine/DMF; (iv) silica gel chromatography/pet. ether/EtOAc; (v) 8 M HCl/dioxan/Δ; (vi) $(MeO)_2CH(CH_2)_2NH_2/\Delta$; (vii) 1 M HCl/Δ; (viii) $NaCNBH_3/NaOAc/AcOH/MeOH$.

Table 5. MRS inhibition and antibacterial activity of enantiomeric tetrahydroquinolines



	Stereochemistry	IC_{50} (nM)	MIC (μg/mL)	
		S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
46	*RS*	11	⩽0.06	⩽0.06
60	*R*	6.3	⩽0.06	⩽0.06
61	*S*	48	16	2

potent antibacterial activity. The compression of IC_{50} values <10 nM due to the limit of the enzyme concentration in the assay (3 nM)[1] makes it hard to calculate the enantiomeric inhibitory ratio. However, the ratio of the antibacterial activity of the two isomers suggests a high degree of enantioselectivity, of the order of at least two orders of magnitude.

In conclusion, the key right hand side pharmacophore for bacterial MRS inhibition has been defined as an NH–C–NH unit in the context of a bicyclic heteroaromatic system. Potent non-quinolone analogues have been obtained with excellent antibacterial activity against staphylococci and enterococci, including antibiotic resistant isolates. In addition, the biologically active configuration of the tetrahydroquinoline series has been identified as possessing (*R*)-stereochemistry.

Acknowledgements

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References and notes

1. Jarvest, R. L.; Berge, J. M.; Berry, V.; Boyd, H. F.; Brown, M. J.; Elder, J. S.; Forrest, A. K.; Fosberry, A. P.; Gentry, D. R.; Hibbs, M. J.; Jaworski, D. D.; O'Hanlon, P. J.; Pope, A. J.; Rittenhouse, S.; Sheppard, R. J.; Slater-Radosti, C.; Worby, A. *J. Med. Chem.* **2002**, *45*, 1959.
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3. Jarvest, R. L.; Berge, J. M.; Brown, P.; Houge-Frydrych, C. S. V.; O'Hanlon, P. J.; McNair, D. J.; Pope, A. J.; Rittenhouse, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1265.
4. The methoxy-thienopyridines were prepared from the 2,4-dichloro precursors by treatment with sodium methoxide. The thieno[3,2-*b*]pyridine isomer gave exclusively the unwanted 2-methoxy isomer unless 15-crown-5 was added, which resulted in an almost complete reversal of specificity (81% 4-methoxy, 3% 2-methoxy). See Ref. 2 for a discussion of a related but less-pronounced case.
5. 2-Chloro-3-cyano-1-(2-trimethylsilylethoxymethyl)-indole was subjected to the following reaction sequence: i. $BocNH(CH_2)_3NH_2/DMSO/\Delta$; ii. TFA/anisole then AcOH; iii. 3,5-diBr-benzyl bromide/K_2CO_3/THF; iv. $LiBF_4$/MeCN/TFA/$CH_2(CH_2SH)_2$.
6. Based on STO-3G ab initio calculations of gas phase energies using AM1 optimised geometries.
7. Colourless needle, 0.38×0.05×0.04 mm, orthorhombic, space group $P2_12_12_1$ (#19), $T = 150$ K, $a = 4.8880(4)$ Å, $b = 13.7295(12)$ Å, $c = 26.441(2)$ Å, $V = 1774.4(3)$ Å^3, $Z = 4$, $D_{calcd} = 1.700$ Mg/m^3, $F(000) = 904$, $\mu(CuK_\alpha)$, $\lambda = 1.54178$ Å) = 5.902 mm^{-1}, Bruker SMART 6000 diffractometer, 11,989 reflections collected ($6.68° \leqslant 2\theta \leqslant 145.52°$), 3430 unique reflections ($R_{int} = 0.0530$), Gaussian absorption correction (transmission = 0.37514–0.80284), full-matrix least-squares refinement (on F^2) of 226 variables, $R_1 = 0.0316$ ($wR_2 = 0.0789$) for 3284 observed data with $I \geqslant 2\sigma(I)$, $R_1 = 0.0327$ ($wR_2 = 0.0805$) for all data, $S = 1.037$, $w = 1/[\sigma^2(F_o^2) + (0.0604P)^2]$ where $P = [\mathrm{Max}(F_o^2, 0) + 2F_c^2]/3$, residual electron density between −0.471 and 0.942 e Å^{-3}, absolute structure parameter = −0.046(19). Crystallographic data for the structure has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 232517.
8. Fused silica 50 cm×50 μm i.d., 20 kV, 100 mM sodium phosphate buffer pH 2.5 containing 40 mM α-cyclodextrin.